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Erythromycin attenuates contractile responses of isolated urinary bladders of hyperthyroid rats

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ABSTRACT

The present study was undertaken to determine the effects of erythromycin on urinary bladder muscle contractions in hyperthyroid rats. Totally 12 rats were assigned into 2 groups (6 rats in each group), as the control and hyperthyroid rat groups. The contractile responses were determined as E_{max} and pD_2 of carbachol (10^{-3}-10^{-8} M) and potassium (1-6×10^{-2} M, KCl) in the absence and presence of erythromycin (10^{-3}; 5×10^{-4}; 10^{-4} M). The contractile responses to carbachol (10^{-3}-10^{-8} M) in the presence of verapamil (10^{-8} M), atropine (10^{-8} M), or in calcium-free Krebs solution were also determined in the absence and presence of erythromycin (10^{-3} M). Treatment of erythromycin significantly reduced the response to carbachol and KCl-evoked contractions. Carbachol-evoked contractions were reduced in the presence of atropine, whereas the atropine-resistant components of carbachol-evoked contractions were not inhibited in the presence of erythromycin. The contractile response to carbachol was reduced in calcium-free Krebs solution and 10^{-8} M verapamil. In addition, when erythromycin was added together with verapamil 10^{-8} M, the contractile response to carbachol was inhibited. In conclusion, erythromycin more effectively inhibited urinary bladder contractions in hyperthyroid rats than the control rats, through calcium movement inhibition.

Key words: erythromycin, hyperthyroid, urinary bladder, contraction, inhibition, rat

Introduction

Thyroid hormones have an important role in the organism in terms of their effects on metabolism, development, thermoregulation, and growth (LAZAR, 1993). In addition to these, various pathological conditions, such as Graves' disease and tumors of the thyroid, produce more hormones, which results in hyperthyroidism (GULCELIK et al., 2006). Increases in the level of these hormones cause not only basal metabolic disorders but also health problems, such as diabetes mellitus and cardiovascular diseases (PANDA and

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KAR, 2007). Hyperthyroidism may also cause dysfunctions of the lower urinary tract (HO et al., 2011) and distressing symptoms such as nocturia, which is a source of significant problems for many patients (WEIN et al., 2002).

The effects of erythromycin, as an antibiotic and prokinetic agent, on smooth muscle contractions have been evaluated comprehensively in many studies (ARMSTRONG et al., 1992; MINOCHA and GALLIGAN, 1991; NISSAN et al., 1997; TAMAOKI et al., 1995). In an experiment with healthy volunteers, dose-dependent erythromycin acts through two different mechanisms: intravenous erythromycin (200 mg) evoked non-propagating, atropine-resistant contractions of the stomach by direct action on the muscle, whereas a lower dose (40 mg) stimulated propagating gastric motility, prevented by atropine (COULIE et al., 1998). In mammals, through experiments with isolated rabbit stomachs, low concentrations of motilin and erythromycin greatly facilitated cholinergically mediated contractions evoked by electrical field stimulation (EFS), whereas higher concentrations contracted the muscle (DASS et al., 2003; JARVIE et al., 2007).

FURNESS et al. (1999) reported that erythromycin lactobionate caused the concentration-dependent inhibition of nerve-mediated contractions on the longitudinal muscle of guinea-pig ileum. In this study, erythromycin inhibited the contractile responses of the guinea-pig ileum induced by EFS and carbachol. At the same time, erythromycin has no action on the motilin receptor of the ileum. Also, NISSAN et al. (2002) suggested that erythromycin antagonized, in a concentration-dependent manner, the direct cholinergic effects on various smooth muscles in the human alimentary tract. DEPOORTERE and PEETERS (1997) characterized the inhibitory effect of erythromycin as mediated through calcium channels, and this effect was not mediated by motilin receptors. ENGLAND et al. (2004) reported that it inhibited the contractile responses of EFS, carbachol, and potassium, but did not notably reduce direct detrusor muscle contractions in rats. Furthermore, EFS-evoked contractions were inhibited by atropine and erythromycin. Erythromycin, together with nifedipine, reduced carbachol responses in a calcium-free Krebs solution. Our study was aimed at determining the effects of erythromycin on the contractile response of isolated hyperthyroid rats' urinary bladders, and to investigate whether erythromycin has more effect on the hyperthyroid rats than the control rats.

Materials and methods

Chemicals. Carbachol, atropine, verapamil, ethylene glycol tetraacetic acid (EGTA), potassium chloride (KCl) and L-thyroxine obtained from Sigma-Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) were used as trial compounds. All the other chemicals and reagents were purchased from commercial suppliers.

Animals and tissue preparation. Twelve adult male Wistar rats (obtained from the Experimental Animal Research and Application Center, Afyonkarahisar, Turkey) of approximately the same age and weighing 180-220 g, were used for the study. The animals were divided into 2 groups as follows; the first group, the control group (n = 6), was fed a

normal rat diet and not treated by any procedure. The second group, the hyperthyroidism group (n = 6), was induced by subcutaneous L-thyroxin (ARAGÃO et al., 2007) at a dose of 50 µg/100g/day for 10 days.

At the end of the experiment, blood samples from each group were collected and transferred into non-heparinised tubes. Serum levels of free and total T_3 and T_4 hormones were analyzed by colorimetric enzyme immunoassay, using individual ELISA kits (DRG International, USA). Then the animals were sacrificed by cervical dislocation and the abdominal cavities were opened. After that the urinary bladders of the rats were removed and immediately transferred to Krebs solution (NaCl 119 mM, KCl 4.4 mM, NaHCO 20 mM, NaH_2PO_4 1.2 mM, $MgCl_2$ 2.5 mM, glucose 11 mM, $CaCl_2$ 2.5 mM, in distilled wåter pH 7.2, aerated with 95% O_2, 5% CO_2 and warmed to 37 ºC). Each urinary bladder was flushed with buffer and mounted in 20 mL organ baths, filled with gassed Krebs solution, and maintained at 37 ºC. The bladders were placed in an organ bath and one end of the bladder muscle was anchored to a stationary clamp and the other end attached to an isometric force transducer (The BioPac system and MP35 acquisition box were used with FDT05 finger transducers). The chambers were prefilled with 15 mL Krebs solution at 37 ºC and constantly bubbled with 95% O_2 and 5% CO_2. The bladder muscle was placed under tension of 0.5 g for 1 hour to equilibrate. Further, the bladder muscles were placed in Krebs solution for 20 minutes' recovery time between the different concentration-response curves.

The pD_2 is defined as the negative logarithm of the EC_{50}. The potency of a drug is generally quantified as the EC_{50}. This designates the concentration of agonist required to provoke a response halfway between the baseline and the maximum response (E_{max}) of a dose-response curve. The value of the highest contractile response (maximal contraction) was taken as 100%, and the percentages of the other contractile responses of the concentrations were calculated. With these calculations, pD_2 and E_{max} values were obtained using "GraphPad Prism version 5 for Windows". Evaluation of the obtained responses was performed by comparing the effects of erythromycin on the pD_2 and E_{max} values of carbachol and KCl.

Erythromycin was dissolved in 0.25% ethanol (ENGLAND et al., 2004). Erythromycin, atropine, carbachol, verapamil, and KCl were prepared in either normal Krebs or calcium-free Krebs solution on the day of the experiment. Calcium-free Krebs solution was prepared without $CaCl_2$ and with the addition of EGTA 10^{-5} M [12]. The experimental protocols were approved by the Animal Care and Use Committee at Afyon Kocatepe University (Ethical number: 2013/263).

Experimental protocol. The effect of erythromycin on carbachol-evoked responses. The control dose response curves of carbachol were obtained and then repeated in the presence of 10^{-3}, 5×10^{-4} and 10^{-4} M erythromycin. 10^{-3}-10^{-8} M carbachol cumulative concentrations were used in the experiments. In these experiments, the effects of erythromycin were determined on the direct contractile response of rat urinary bladders.

The effect of atropine on carbachol-evoked responses in the absence and presence of erythromycin. Control carbachol dose response curves (10^{-3}-10^{-8}M) were obtained and then repeated after incubation with erythromycin 10^{-3} M for 10 minutes each. Further responses were obtained after incubation with 10^{-8} M atropine alone and then with 10^{-8} M atropine plus 10^{-3} M erythromycin.

The effect of erythromycin on potassium-evoked responses. To determine the effect of erythromycin on the contractile response to potassium, a control contractile response curve was obtained from KCl, 10^{-2} -6×10^{-2} M and then repeated after the bladders were treated with erythromycin (10^{-3}; 5×10^{-4}; 10^{-4} M) in an organ bath including Krebs solution for 10 minutes.

The effect of verapamil on carbachol-evoked responses in the absence and presence of erythromycin. The effect of erythromycin on the contractile response to carbachol was determined in the presence of erythromycin and verapamil. Contractile responses of control were obtained from carbachol (10^{-3}-10^{-8} M) and then repeated after incubation with Krebs and 10^{-8} M verapamil alone, or with 10^{-8} M verapamil plus erythromycin 10^{-3} M.

The effects of erythromycin and verapamil on carbachol-evoked responses in normal and calcium-free Krebs solution. To determine the effect of erythromycin on intracellular calcium release, carbachol-evoked contractile responses were determined in calcium-free Krebs solution. Control contractile responses to carbachol (10^{-3}-10^{-8} M) were determined in normal Krebs solution and then in Ca^{2+} free Krebs solution. The evoked responses to carbachol in calcium-free Krebs solution were obtained following the disappearance of spontaneous contractions after the Krebs solution was changed from that containing calcium to calcium-free. The bladders were then reincubated with normal Krebs solution for 10 minutes to permit the intracellular calcium stores to be replenished. The normal Krebs solution was then switched to calcium-free Krebs solution and the samples were incubated with either 10^{-8} M verapamil alone or with 10^{-8} M verapamil plus erythromycin 10^{-3} M, before being stimulated once more with 10^{-3}-10^{-8} M carbachol, when the spontaneous contractions had ceased.

Statistical analyses. Statistical calculations and graphs were done with "GraphPad Prism version 5 for Windows" (GRAPHPAD, 2010). The results are presented as mean ± SD. Statistical analyses were carried out using one-way analysis of variance followed by Bonferroni's correction for multiple comparisons. Also, comparisons of control and hyperthyroid groups were analyzed with the student *t*-test. Differences were considered significant when the P value was <0.05.

Results

Serum T_3 and T_4 analyses. Free and total T_3 and T_4 levels are shown in Table 1. In our study, the values of these parameters were found to be higher in the hyperthyroid group compared to the control ($P<0.001$).

The effect of erythromycin on carbachol-evoked responses. Erythromycin 10^{-3} M ($P<0.001$) and 5×10^{-4} M ($P<0.01$) reduced the E_{max} responses of the bladders to carbachol in the control group (Table 2). In addition, erythromycin 10^{-3} M, 5×10^{-4} M and 10^{-4} M ($P<0.001$) reduced the E_{max} responses of the bladders to carbachol in the hyperthyroid group (Table 2). Carbachol E_{max} responses in the presence of 5×10^{-4} M and 10^{-4} M erythromycin were found to be lower in the hyperthyroid group compared to the control ($P<0.001$). Also, erythromycin did not have any effect on pD_2 responses of the bladders to carbachol in either group. The dose-response curve of carbachol alone or in the presence of erythromycin is also shown in Fig. 1A and B.

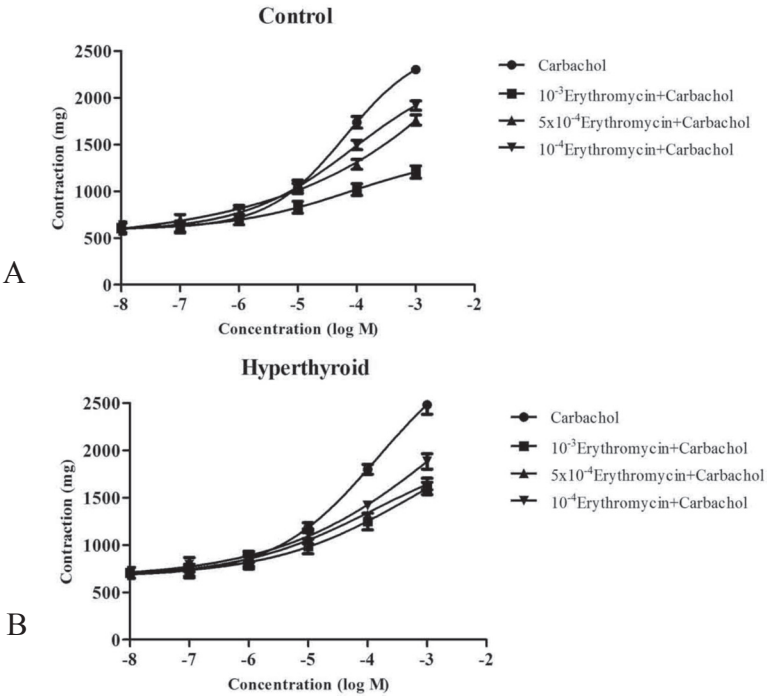

Fig. 1. The concentration-contraction relationships of carbachol treatment (10^{-3}-10^{-8} M) alone and with erythromycin (10^{-3}; 5×10^{-4}; 10^{-4} M) in control (A) and hyperthyroid rats (B). After observing the control responses to carbachol (10^{-3}-10^{-8} M), the urinary bladders were treated with erythromycin (10^{-3}; 5×10^{-4}; 10^{-4} M) for 10 min. Carbachol was applied in the presence of the drug, and the contractile responses to carbachol were compared with the responses in the control. Ordinate: the amplitude of the contraction was expressed as mg of carbachol (10^{-3}-10^{-8} M)-induced contraction. Abscissa: the concentration of drugs (log M). Each point represents the mean of six experiments with SD shown by a vertical line.

Table 1. Free (F) T_3 and T_4 or Total (T) T_3 and T_4 levels in serum samples of control and hyperthyroid rats

Groups (n:6)	FT_3 (pg/dL)	FT_4 (ng/dL)	TT_3 (ng/dL)	TT_4 (µg/dL)
Control	29.9 ± 5.1	0.5 ± 0.1	26.7 ± 4.0	4.2 ± 0.7
Hyperthyroid	56.4 ± 7.4***	1.2 ± 0.1***	128.9 ± 3.2***	6.9 ± 1.2***

Values in the same column marked with stars show statistically significant differences ***P<0.001. Data are expressed as the means ± SD.

Table 2. The E_{max} and pD_2 responses of carbachol and carbachol plus 10^{-3}, 5×10^{-4}, and 10^{-4} M erythromycin on urinary bladders in control and hyperthyroid rats

Treatment (n:6)		Control	Hyperthyroid	P value
Carbachol cumulative	E_{max}	101.6 ± 3.4	103.5 ± 6.1	0.501
	pD_2	5.2 ± 1.3	5.7 ± 0.8	0.843
Erythromycin 10^{-3} M + Carbachol cumulative	E_{max}	66.6 ± 9.2***	62.4 ± 7.2***	0.408
	pD_2	4.8 ± 0.4	3.6 ± 1.6	0.830
Erythromycin 5×10^{-4} M + Carbachol cumulative	E_{max}	86.5 ± 7.4**	60.4 ± 7.4***	0.000
	pD_2	4.7 ± 0.8	4.0 ± 1.3	0.269
Erythromycin 10^{-4} M + Carbachol cumulative	E_{max}	99.5 ± 1.3	77.9 ± 8.6***	0.000
	pD_2	5.0 ± 1.5	4.8 ± 0.8	0.016

In the same row values with P value and in the same column values with stars show statistically significant differences **P<0.01; ***P<0.001. In the presence of erythromycin, carbachol E_{max} and pD_2 levels were compared with carbachol cumulative levels. Data are expressed as the means ± SD.

Table 3. The E_{max} and pD_2 responses of carbachol and carbachol plus 10^{-3} M erythromycin or 10^{-8} M atropine on urinary bladders in rats

Treatment (n:6)		Control	Hyperthyroid	P value
Carbachol cumulative	E_{max}	101.5 ± 3.4	103.5 ± 6.1	0.501
	pD_2	5.7 ± 1.0	5.2 ± 1.2	0.477
Atropine 10^{-8} M + Carbachol cumulative	E_{max}	87.9 ± 4.9***	83.3 ± 3.3***	0.088
	pD_2	4.8 ± 0.4	4.5 ± 1.4	0.713
Erythromycin 10^{-3} M + Atropine 10^{-8} M + Carbachol cumulative	E_{max}	86.5 ± 3.8***	84.1 ± 4.1***	0.314
	pD_2	4.7 ± 0.4	4.5 ± 0.4	0.238

In the same row values with P value and in the same column values with stars show statistically significant differences ***P<0.001. In the presence of atropine and atropine plus erythromycin, carbachol E_{max} and pD_2 levels were compared with carbachol cumulative levels. Data are expressed as the means ± SD.

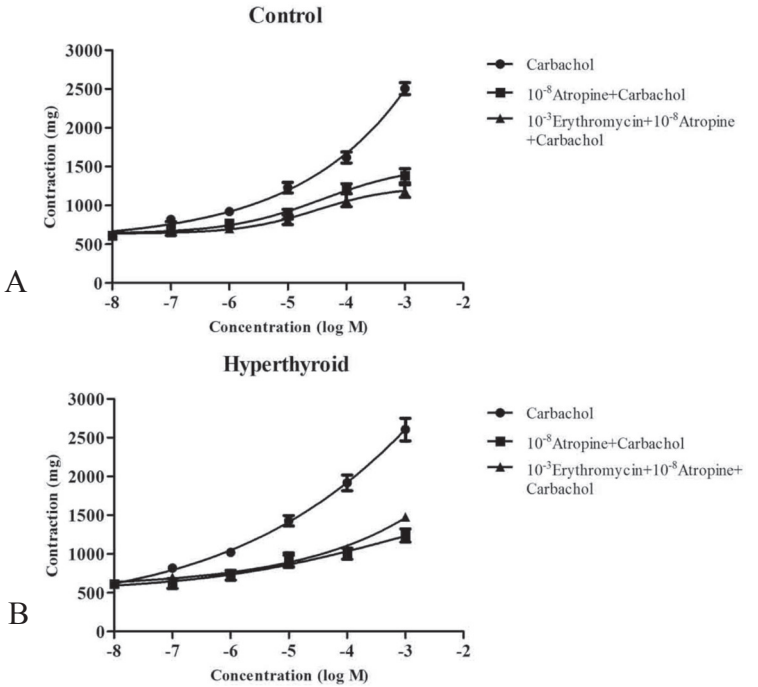

Fig. 2. The concentration-contraction relationships of carbachol treatment (10^{-3}-10^{-8} M) alone or with erythromycin (10^{-3} M) and atropine (10^{-8} M) in control (A) and hyperthyroid rats (B). After observing the control responses to carbachol (10^{-3}-10^{-8} M), the urinary bladders were treated with erythromycin (10^{-3}; 5×10^{-4}; 10^{-4} M) or atropine (10^{-8} M) for 10 min. Carbachol was applied in the presence of these drugs and the contractile responses to carbachol were compared with the responses in the control. Ordinate: the amplitude of the contraction was expressed as mg of carbachol (10^{-3}-10^{-8} M)-induced contraction. Abscissa: the concentration of drugs (log M). Each point represents the mean of six experiments with SD shown by a vertical line.

The effect of atropine on carbachol-evoked responses in the absence and presence of erythromycin. Incubation of bladders with 10^{-8} M atropine and 10^{-8} M atropine plus 10^{-3} M erythromycin reduced the carbachol E_{max} compared to carbachol E_{max} alone ($P<0.001$). Additionally, 10^{-8} M atropine plus 10^{-3} M erythromycin did not show any significant difference in carbachol E_{max} compared to carbachol E_{max} in the presence of 10^{-8} M atropine (Table 3). 10^{-8} M atropine alone and the 10^{-8} M atropine plus 10^{-3} M erythromycin treatment reduced carbachol responses as shown in Fig. 2A and B. Erythromycin had no significant effect on the pD_2 responses of rat bladders to carbachol in either group.

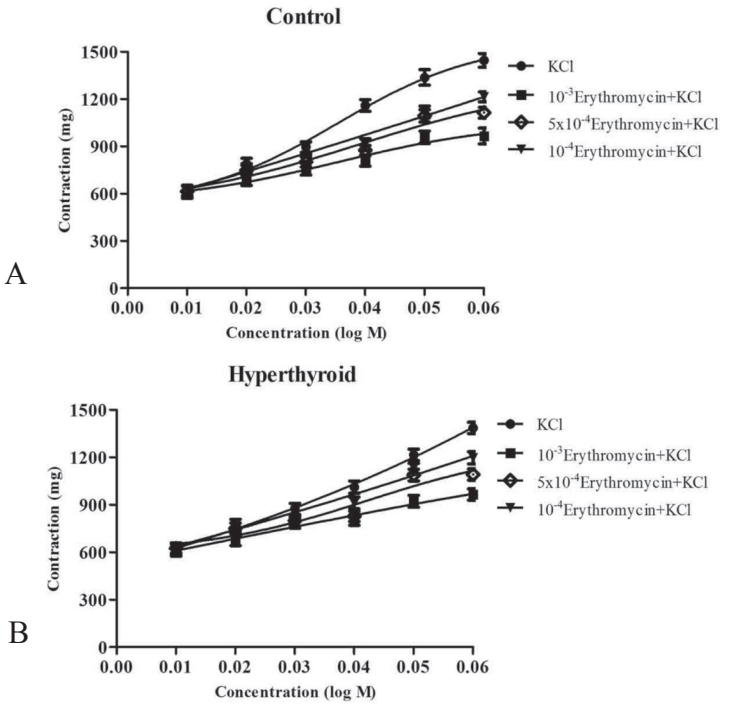

Fig. 3. The concentration-contraction relationships of potassium chloride treatment (KCl; 1-6×10^{-2} M) alone and with erythromycin (10^{-3}; 5×10^{-4}; 10^{-4} M) in control (A) and hyperthyroid rats (B). After observing the control responses to KCl (1-6×10^{-2} M), the urinary bladders were treated with erythromycin (10^{-3}; 5×10^{-4}; 10^{-4} M) for 10 min. KCl was applied in the presence of the drug and the contractile responses to KCl were compared with the responses in the control. Ordinate: the amplitude of the contraction was expressed as mg of KCl (1-6×10^{-2} M)-induced contraction. Abscissa: the concentration of drugs (log M). Each point represents the mean of six experiments with SD shown by a vertical line.

Table 4. The E_{max} and pD_2 responses of 1×10^{-6} - 6×10^{-6} M KCl and KCl plus 10^{-3}, 5×10^{-4}, and 10^{-4} M erythromycin on urinary bladders in rats

Treatment (n:6)		Control	Hyperthyroid	P value
KCl cumulative	E_{max}	99.1 ± 4.1	94.8 ± 4.3	0.104
	pD_2	2.1 ± 0.2	2.1 ± 0.4	0.283
Erythromycin 10^{-3} M + KCl cumulative	E_{max}	90.4 ± 1.8**	85.2 ± 2.7***	0.003
	pD_2	1.8 ± 0.2	1.8 ± 0.3	0.126
Erythromycin 5×10^{-4} M + KCl cumulative	E_{max}	91.3 ± 3.1**	86.7 ± 2.9**	0.029
	pD_2	1.8 ± 0.5	1.8 ± 1.0	0.122
Erythromycin 10^{-4} M + KCl cumulative	E_{max}	93.4 ± 4.2	89.6 ± 2.9	0.094
	pD_2	1.8 ± 1.0	1.9 ± 0.2	0.808

In the same row values with P value and in the same column values with stars show statistically significant differences **P<0.01; ***P<0.001. In the presence of erythromycin, KCl E_{max} and pD_2 levels were compared with carbachol cumulative levels. Data are expressed as the means ± SD.

Table 5. The E_{max} and pD_2 responses of carbachol and carbachol plus 10^{-3} M erythromycin and 10^{-8} M verapamil on rat urinary bladder in normal and calcium-free Krebs solution

Treatment (n:6)		Control	Hyperthyroid	P value
Carbachol cumulative	E_{max}	101.5 ± 3.4	103.5 ± 6.1	0.501
	pD_2	4.8 ± 0.4	4.8 ± 0.8	0.843
Verapamil 10^{-8} M + Carbachol cumulative	E_{max}	89.9 ± 2.1***	89.3 ± 2.7***	0.355
	pD_2	4.3 ± 0.7	4.2 ± 0.5	0.371
Erythromycin 10^{-3} M + Verapamil 10^{-8} M + Carbachol cumulative	E_{max}	82.6 ± 2.3***	84.3 ± 2.6***	0.086
	pD_2	3.9 ± 0.6	3.9 ± 0.5	0.135
Carbachol cumulative in Calcium-free Krebs	E_{max}	92.2 ± 3.1**	93.6 ± 2.7***	0.435
	pD_2	3.5 ± 0.5*	3.5 ± 0.5**	0.467
Verapamil 10^{-8} M + Carbachol cumulative in Calcium-free Krebs	E_{max}	80.0 ± 4.1***	84.1 ± 3.7***	0.280
	pD_2	3.1 ± 0.6***	3.1 ± 0.5***	0.587
Erythromycin 10^{-3} M + Verapamil 10^{-8} M + Carbachol cumulative in Calcium-free Krebs	E_{max}	71.2 ± 6.6***	73.4 ± 3.2***	0.016
	pD_2	2.8 ± 0.6***	2.6 ± 0.2***	0.835

In the same row values with P value and in the same column values with stars show statistically significant differences *P<0.05; **P<0.01; ***P<0.001. In the presence of verapamil or Ca-free verapamil alone and together with erythromycin, carbachol E_{max} and pD_2 levels were compared with carbachol cumulative levels. Data are expressed as the means ± SD.

The effect of erythromycin on potassium-evoked responses. 10^{-3} M and 5×10^{-4} M erythromycin ($P<0.01$) reduced the E_{max} responses of bladders to KCl in the control group (Table 4). Similarly, 10^{-3} M ($P<0.001$) and 5×10^{-4} M ($P<0.01$) erythromycin reduced the E_{max} responses of bladders to KCl in the hyperthyroid group (Table 4). KCl E_{max} responses in the presence of 5×10^{-4} M and 10^{-4} M erythromycin were found to be lower in the hyperthyroid group compared with the control ($P<0.05$). The dose-response curves of KCl alone or in the presence of erythromycin are shown in Fig. 3A and B. Erythromycin had no significant effect on the pD_2 responses of rat bladders to KCl in either group.

The effect of verapamil on carbachol-evoked responses in the absence and presence of erythromycin. To determine the effect of erythromycin on calcium release from intracellular stores, responses to carbachol were obtained in the presence of verapamil, a potent inhibitor of Ca^{2+} influx through L-type (REUTER, 1983; INCE and FILAZI, 2009). The addition of 10^{-8} M verapamil to the organ bath reduced the E_{max} compared to carbachol control responses ($P<0.001$) in the control and hyperthyroid groups. The further addition of erythromycin 10^{-3} M reduced E_{max} compared to the carbachol control responses ($P<0.001$) in the control and hyperthyroid groups (Table 5). The effects of 10^{-8} M verapamil and 10^{-3} M erythromycin treatment on carbachol responses are shown in Fig. 4A and B. Erythromycin had no significant effect on the pD_2 responses of rats' bladders to carbachol in either group.

The effect of erythromycin and verapamil on carbachol-evoked responses in calcium-free Krebs solution. The experiment described above was repeated in calcium-free Krebs solution to further assess the effect of erythromycin on intracellular calcium stores. A lower concentration of 10^{-8} M verapamil was used to reduce the possibility of non-specific effects, with a shorter incubation period (until spontaneous contractions ceased) to minimize the loss of calcium from intracellular stores. The contractile responses of bladders to carbachol in calcium-free Krebs solution inhibited the E_{max} of the control ($P<0.01$) and hyperthyroid ($P<0.001$) groups, and the pD_2 of the control ($P<0.05$) and hyperthyroid ($P<0.01$) groups, compared to the control responses in normal Krebs solution. Addition of 10^{-8} M verapamil to calcium-free Krebs solution had an additional inhibitory effect on the E_{max} and pD_2 responses to carbachol. When erythromycin 10^{-3} M was added with 10^{-8} M verapamil it inhibited carbachol E_{max} and pD_2 responses compared to calcium-free+10^{-8} M verapamil-carbachol responses (Table 5), and the groups' responses are shown in Fig. 4A and B.

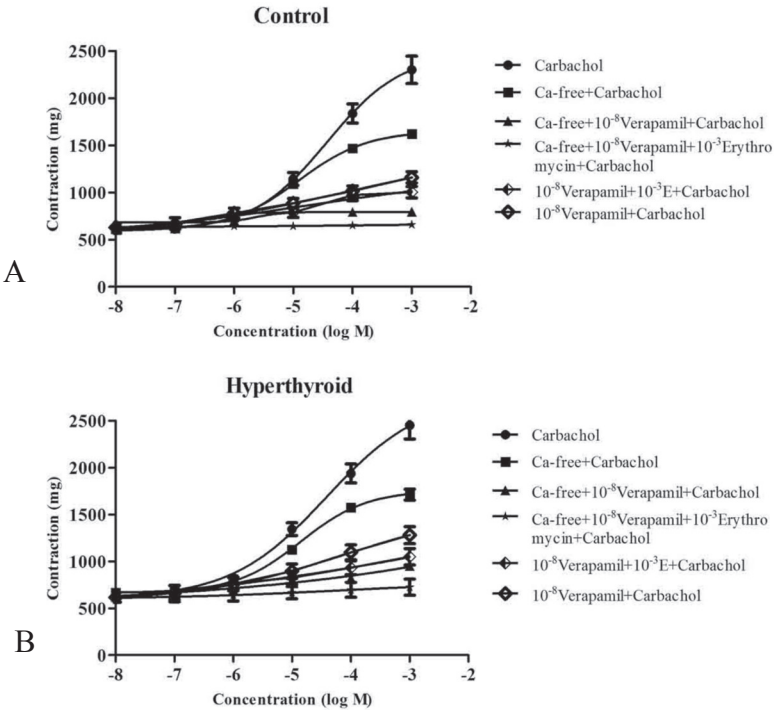

Fig. 4. The concentration-contraction relationships of carbachol treatment (10^{-3}-10^{-8} M) alone or with erythromycin (10^{-3} M) and verapamil (10^{-8} M) in normal and Ca-free Krebs solutions of control (A) and hyperthyroid rats (B). After observing the control responses to carbachol (10^{-3}-10^{-8} M), the urinary bladders were treated with verapamil (10^{-8} M) or Ca-free verapamil (10^{-8} M) alone and together with erythromycin (10^{-3} M) for 10 min. Carbachol was applied in the presence of these drugs and the contractile responses to carbachol were compared with the responses in the control. Ordinate: the amplitude of the contraction was expressed as mg of carbachol (10^{-3}-10^{-8} M)-induced contraction. Abscissa: the concentration of drugs (log M). Each point represents the mean of six experiments with SD shown by a vertical line.

Discussion

The present study showed that erythromycin had a more potent ability to inhibit carbachol and KCl contractions in hyperthyroid rats than in control rats. Carbachol is a cholinomimetic agent and has an important role in smooth muscle contraction (MOKRY et al., 2002). In this study, erythromycin (10^{-3}; 5×10^{-4}; 10^{-4} M) treatment affected the E_{max} of carbachol in the hyperthyroid rats. This suggests that the urinary bladder of hyperthyroid rats has a more sensitive response and erythromycin could be more potent in hyperthyroid

rats. Pretreatment with 10^{-8} M atropine attenuated carbachol contractions in both groups. However, 10^{-3} M erythromycin treatment did not change the atropine-resistant response to carbachol, indicating that erythromycin may not affect the muscarinic receptors binding carbachol in the urinary bladder.

KCl may depolarize the cellular membrane and activate the calcium channel, resulting in calcium inflow and then smooth muscle contraction (VAN et al., 1980). Erythromycin inhibited KCl response and it was concluded that the effect of erythromycin on the urinary bladder is related to Ca^{2+}, and affects the Ca^{2+} pathway. Also, erythromycin (10^{-3} and 5×10^{-4} M) treatment reduced the KCl contractions induced by cumulative concentrations (10^{-2} - 6×10^{-2} M) in the urinary bladders of hyperthyroid rats more than in normal rats, due to the failing function of the urinary bladder.

Verapamil is an L-type calcium channel antagonist, and inhibits smooth muscle contractions by inhibiting Ca^{2+} influx via the calcium channel. Correspondingly, erythromycin acts as a Ca^{2+} channel antagonist and inhibited the extracellular Ca^{2+} influx through the calcium channel (ENGLAND et al., 2004). Similarly, in our study, a sub-maximal verapamil dose reduced carbachol E_{max} levels in the urinary bladder. 10^{-3} M erythromycin and verapamil treatment slightly reduced E_{max} levels of carbachol obtained with verapamil in the urinary bladders of all the rats. As is known, Ca^{2+} has an important role in smooth muscle contraction. Ca^{2+} concentration is increased in the cytoplasm, causing smooth muscle contraction. This comes from sarcoplasmic reticulum (SR) release or influx, via different Ca^{2+} channels. Also, Ca^{2+} is mainly stored in the SR of smooth muscle cells. In addition, extracellular Ca^{2+} influx is a good way of maintaining continuous smooth muscle contractions and restoring the Ca^{2+} pool (KONG et al., 2006). To investigate the possible effects, we investiged the 10^{-3} M erythromycin carbachol-evoked contractile responses of urinary bladders on normal and hyperthyroid rats perfused with calcium free Krebs solution including verapamil, to remove the calcium entry component. Contractions were reduced in the absence of extracellular calcium, and inhibited further by the calcium entry blocker, verapamil, indicating residual contractile activity in these experiments. The addition of 10^{-3} M erythromycin to calcium free and verapamil-containing Krebs solution inhibited the E_{max} and pD_2 levels of carbachol, suggesting inhibitory activity on the release of intracellular calcium or alteration of the calcium contractile mechanism within the cells. This suggested that erythromycin correspondingly inhibited both the release of intracellular calcium and extracellular Ca^{2+} influx in normal and hyperthyroid rats, and similarly to studies undertaken by other researchers (NISSAN et al., 1999; ENGLAND et al., 2004), it reduced muscle contractions by calcium influx-efflux. Also, erythromycin affected the urinary bladder contractions of hyperthyroid rats more due to bladder dysfunction.

To date, the pharmacological effects of erythromycin have been demonstrated by many studies (ARMSTRONG et al., 1992; MINOCHA and GALLIGAN, 1991; NISSAN et al., 1997; TAMAOKI et al., 1995; NISSAN et al., 1999; PEETERS et al., 1989), for example on erythromycin inhibited ileum contractions in guinea pigs (NISSAN et al., 1999), and smooth muscle gallbladder contractions in guinea pigs (NISSAN et al., 1997) and humans (TAMAOKI et al., 1995). However, erythromycin increased motility in the terminal rabbit ileum by the activation of dihydropyridine-sensitive calcium channels (ARMSTRONG et al., 1992). Also, DEPOORTERE and PEETERS (1997) reported that erythromycin reduced gastrointestinal smooth muscle contractions in rabbits, and this inhibitory effect of erythromycin was mediated by means of calcium channels, thereby reducing calcium influx.

In conclusion, the results show that erythromycin has an effect on urinary bladder contractions induced by carbachol or KCl and also their contractile effects are more affected by erythromycin in hyperthyroid rats than in normal rats. For these reasons, uptake and elimination of erythromycin may result in reduced contractility of the urinary bladder in hyperthyroidism.

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Conflict of interest

The author(s) confirm that this article content involves no conflict of interest.

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SAŽETAK

Istraženi su učinci eritromicina na kontrakcije mišića mokraćnog mjehura u hipertireotičnih štakora. Dvanaest štakora bilo je svrstano u dvije skupine, kontrolnu i pokusnu (po šest štakora u svakoj skupini). Kontraktilni odgovor izražen kao E_{max} i pD_2 na karbakol (10^{-3}-10^{-8} M) i kalij (1-6×10^{-2} M, KCl) bili su određeni u odsutnosti i prisutnosti eritromicina (10^{-3}; 5×10^{-4}; 10^{-4} M). Kontraktilni odgovori na karbakol (10^{-3}-10^{-8} M) u prisutnosti verapamila (10^{-8} M), atropina (10^{-8} M), ili u Krebsovoj otopini bez kalcija, bili su također određivani u odsutnosti i prisutnosti eritromicina (10^{-3} M). Primjena eritromicina značajno je smanjila odgovor na kontrakciju potaknutu karbakolom i kalijevim kloridom. Kontrakcije potaknute karbakolom bile su smanjene u prisutnosti atropina, dok komponente kontrakcija izazvane karbakolom rezistentne na atropin nisu bile inhibirane u prisutnosti eritromicina. Kontraktilni odgovor na karbakol bio je smanjen u Krebsovoj otopini bez kalcija i 10^{-8} M otopini verapamila. Povrh toga, kontraktilni odgovor na karbakol bio je inhibiran kad je eritromicin bio primijenjen zajedno s 10^{-8} M verapamilom. Može se zaključiti da je eritromicin učinkovitije inhibirao kontrakcije mokraćnog mjehura hipertireotičnih štakora nego u kontrolnih štakora putem inhibicije kolanja kalcija.

Ključne riječi: eritromicin, hipertireoidizam, mokraćni mjehur, kontrakcija, inhibicija, štakor